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Hot Topic

ESR1 mutations: Moving towards guiding treatment decision-making in metastatic breast cancer patients

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ABSTRACT

Mutations in the gene coding for the estrogen receptor (ER), *ESR1*, have been associated with acquired endocrine resistance in patients with ER-positive metastatic breast cancer (MBC). Functional studies revealed that these *ESR1* mutations lead to constitutive activity of the ER, meaning that the receptor is active in absence of its ligand estrogen, conferring resistance against several endocrine agents. While recent clinical studies reported that the occurrence of *ESR1* mutations is rare in primary breast cancer tumors, these mutations are more frequently observed in metastatic tissue and circulating cell-free DNA of MBC patients pretreated with endocrine therapy. Given the assumed impact that the presence of *ESR1* mutations has on outcome to endocrine therapy, assessing *ESR1* mutations in MBC patients is likely to be of significant interest to further individualize treatment for MBC patients. Here, *ESR1* mutation detection methods and the most relevant pre-clinical and clinical studies on *ESR1* mutations regarding endocrine resistance are reviewed, with particular interest in the ultimate goal of guiding treatment decision-making based on *ESR1* mutations.

Introduction

Endocrine therapy with selective estrogen receptor modulators/downregulators (SERMs/SERDs) or by estrogen deprivation using aromatase inhibitors (AIs), is the most important treatment modality for estrogen receptor (ER)-positive metastatic breast cancer (MBC) patients [1]. Unfortunately, 40% of patients do not benefit from first-line endocrine therapy due to intrinsic resistance, and the remainder of patients initially responding will eventually develop resistance during therapy [1]. Several mechanisms have been linked to endocrine resistance, however, no marker for resistance has reached wide clinical use yet [2–4]. Recently, mutations in the gene encoding ERα, *ESR1*, have attracted particular interest as a mechanism for endocrine resistance in MBC. Large-scale next-generation sequencing (NGS) efforts on MBC tissues revealed that these mutations are enriched in MBC patients treated with endocrine agents while these variants are not or only at very low frequencies present in primary tumor tissue [5,6]. Importantly, this implies that their presence has to be assessed in metastatic lesions, or in "liquid biopsies" such as circulating cell-free DNA (cfDNA) as a representative of metastatic tumor cells. Here we review the pros and cons of current detection methods for *ESR1* mutations, the preclinical and clinical studies investigating *ESR1* mutations and highlight its potential role in treatment decision-making in MBC patients.

Functional studies on ESR1 mutations

The ER belongs to the nuclear hormone receptor superfamily [7] and consists of two activation function (AF)-1/2 domains, DNA binding and hinge domains, and a ligand binding domain (LBD) (Fig. 1). The ER functions as a ligand-dependent transcription factor. Binding of estradiol to the LBD leads to a conformational change of helix 12, resulting in recruitment of coregulatory proteins [8]. This eventually yields transcription of genes important in normal physiological processes but also for breast tumorigenesis and breast cancer (BC) progression [9].

Recent NGS efforts revealed that somatic *ESR1* mutations in the LBD were more frequently present in metastatic lesions than previously thought. In preclinical models to evaluate the role of *ESR1* mutations in endocrine resistance, it was demonstrated that cell lines transfected with a D538G, Y537S, L536Q, Y537N, Y537C, S463P or E380Q *ESR1* mutation exert activity in the absence of estrogen [6,10–15] (Fig. 1). This constitutive activity suggests that estrogen-depriving therapies such as AIs are not or less effec-

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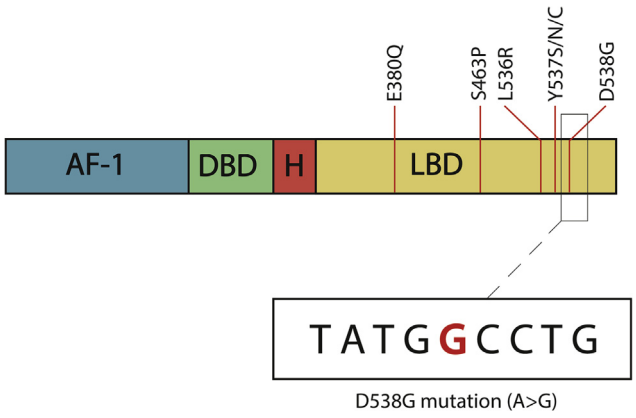

Fig. 1. Schematic overview of the different domains of the ER. Activation function (AF) domain-1 present at the N-terminus acts in a ligand-independent manner, whereas, the AF-2 within the ligand binding domain (LBD) is dependent on estradiol for its activation [52]. The DNA binding domain encodes two zinc finger molecules, playing an important role in receptor dimerization and binding of the ER to specific DNA sequences: the estrogen response element (ERE) [53]. H = hinge region. *ESR1* mutations, some hotspot mutations shown as vertical red lines, mainly occur in the C-terminal domain of the receptor encoding for the LBD of the ER.

tive in patients with activating *ESR1* mutations. Cell lines transfected with mutant *ESR1* variants were however still responsive to treatment with tamoxifen and fulvestrant, though sensitivity to these drugs was relatively impaired compared to *ESR1* wildtype transfected cell lines [5,6,12,13]. Similar observations were recently made for novel SERM/SERD hybrid endocrine therapies pipendoxifene and bazedoxifene [16].

Techniques to detect ESR1 mutations

Several techniques can be used to assess *ESR1* mutations in tissue or cfDNA (Fig. 2), all having their own advantages and disadvantages. Importantly, these techniques widely vary in their sensitivity. NGS can be performed either in the context of whole genome sequencing, as part of a whole exome panel, or as part of a targeted *ESR1* panel. While NGS is an established and widely used approach for mutation detection in tumor tissue, mutation detection in cfDNA is more challenging, as the relative number of mutant to wildtype DNA alleles has to be taken into account. Frequencies of circulating tumor DNA (ctDNA) vary largely between patients, frequently being below 1% of the total cfDNA [17], which is beyond the sensitivity of conventional NGS. Therefore, techniques based on digital PCR (dPCR) have been introduced enabling the detection of ctDNA in frequencies as low as 0.001% [18,19]. In dPCR-based techniques, each individual DNA molecule, within its own partition, is able to react with a specific probe for wildtype *ESR1* and another probe for a specific *ESR1* mutant. There are several commercially available dPCR-based assays (e.g. digital PCR, droplet digital PCR (ddPCR), BEAMing), differing in used reagents and sample readouts, but generally having similar sensitivity [17,20]. In a study comparing conventional targeted NGS with dPCR to detect mutations in cfDNA, threefold more D538G *ESR1* mutations in cfDNA were observed using dPCR than with NGS [21]. One disadvantage of dPCR assays is however that only a subset of hotspot mutations can be evaluated. Other assays, using some sort of target-enrichment prior to analysis, can be used to detect multiple hotspot mutations (OnTarget assay [22,23]) or multiple frequently mutated genes (e.g. SafeSeqS [24], CAPP-Seq [25]), however to date these assays have not yet been reported to be used to detect *ESR1* mutations.

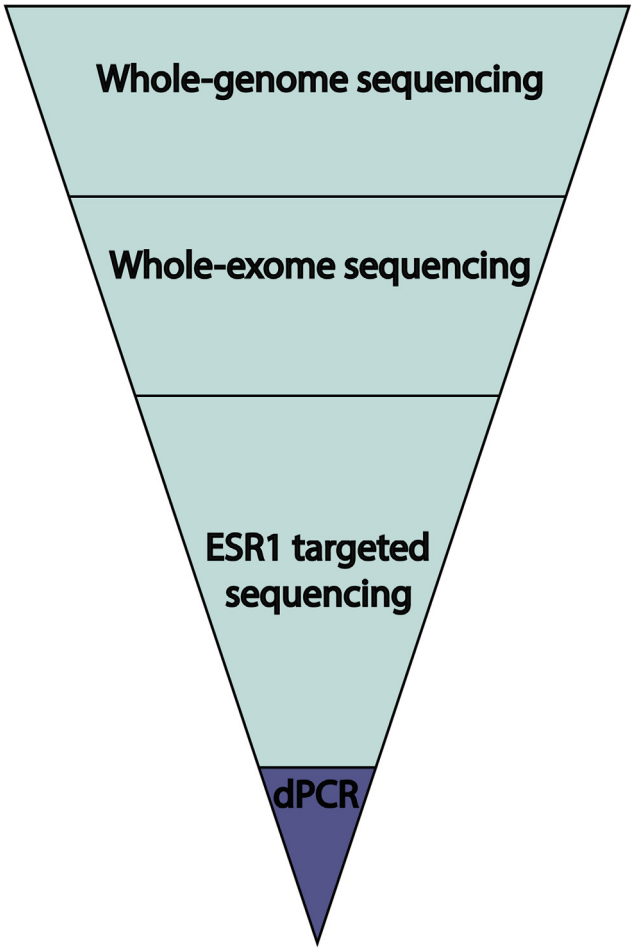

Fig. 2. Various techniques for *ESR1* mutation detection. The pyramid represents the range in which the genome is investigated. *ESR1* mutations can be detected by large-scale NGS efforts such as whole-genome sequencing or whole-exome sequencing, or by more targeted methods as targeted sequencing of the *ESR1* gene only, or by the interrogation of individual mutations in *ESR1* by digital PCR.

Clinical studies on the significance of *ESR1* mutations

ESR1 mutations in primary and metastatic tumor tissue

Although already described anecdotally in the the '90s [11,26,27], *ESR1* mutations were thought to be rare in BC. They occur only in up to 3% of primary tumors using NGS (Supplementary Table 1) [5,6,12,13]. Using more sensitive dPCR-based techniques, the *ESR1* mutation rate in primary BC tumors may mildly increase [28,29], however, only at very low variant allele frequencies (VAF; 0.07–0.2%) [29].

In contrast to mutation rates in primary BC, the landmark papers of Toy et al. [6] and Robinson et al. [13] showed much higher *ESR1* mutation rates in metastatic lesions (Supplementary Table 2). Toy and colleagues [6] found *ESR1* mutations (predominantly D538G and Y537S) in metastatic tissues in 9/36 ER-positive MBC patients who had received at least 3 months of endocrine therapy. All patients with an *ESR1* mutation were at least treated with two lines of endocrine therapy; all containing an AI. In an independent cohort of 44 metastatic tumors from patients pretreated with endocrine therapy, 5 metastases (11%) harbored an *ESR1* mutation.

Likewise, Robinson et al. [13] demonstrated *ESR1* mutations in 6/11 (55%) evaluated metastatic biopsies of ER-positive MBC patients. All patients with an *ESR1* mutation were pretreated with